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Codon 4 ACT→ACA, Codon 5 CCT→TCT, and Codon 6 GAG→TAG Mutations in cis Position: A Form of Thalassemia Trait

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A female of Uttar Pradesh, of Indian origin, who had a transfusion-dependent child, carried codon 4 ACT→ACA, codon 5 CCT→TCT, and codon 6 GAG→TAG mutations at the cis position. The mutation was detected through sequencing of the amplified β-globin gene. Heterozygosity is expressed as a thalassemia trait with moderate anemia, low MCV (57 fl), raised HbA_2 (6.7%), and normal fetal hemoglobin (1.4%). Am. J. Hematol. 56:187–188, 1997. © 1997 Wiley-Liss, Inc.

Key words: ARMS; β-globin gene; thalassemia trait; mutation; sequencing

INTRODUCTION

The term ''thalassemia trait'' is used to identify a large group of β-thalassemia carriers which result either in no anemia or moderate anemia requiring no blood transfusion. Clinical features are essentially normal. In Cooley's anemia or thalassemia major, both chromosomes carry the mutation, thus leading to transfusion-dependence, whereas with thalassemia traits, only one chromosome is affected and blood transfusion is not required.

This report describes an Indian female of Uttar Pradesh with a classical β-thalassemia trait, i.e., raised HbA_2 (6.7%), decreased osmotic fragility (27.5%), and low MCV (57 fl), with a combination of point mutations at cis position.

MATERIALS AND METHODS

A 3-year-old Thakur male child from Uttar Pradesh was referred to us for management of severe anemia. Blood samples of the proband and his parents were collected for thalassemia screening. Red blood cell indices were worked out on a Sysmex 800 blood cell counter. Fetal hemoglobin estimation was done by alkali denaturation test [1], and HbA_2 was estimated by the method described by Schleider et al. [2]. Hemoglobin electrophoresis was carried out on cellogel in Trisglycine buffer at pH 8.9. Serum iron and total iron-binding capacity was estimated to rule out the possibility of iron-deficiency anemia.

DNA was isolated from white cells using the method described by Poncz et al. [3]. ARMS technique, as described by Newton et al. [4], was used for characterization of β-thalassemia mutations, and primers, as described by Varawalla et al. [5], were used for common β-thalassemia mutations. The whole of the β-globin gene was amplified by PCR, which was further amplified in four parts for SSCP [6] (silver stained [7]). A portion of an abnormal band separated on SSCP was picked up by Eppendorf pipette tip, reamplified, and subjected to non-radioactive direct sequencing (sequencing high-cycle, Toyobo).

RESULTS

The subject belongs to Uttar Pradesh, a North Indian state. Hematological investigations of the proband and his parents are shown in the pedigree in Figure 1.

On screening for common β-thalassemia mutations described in Indians, the father and proband were found positive for the IVS 1–5 (G→C) mutation, while the mother tested negative for all the mutations described so far. Since the proband was transfusion-dependent and undergoing iron chelation therapy regularly, the mother's

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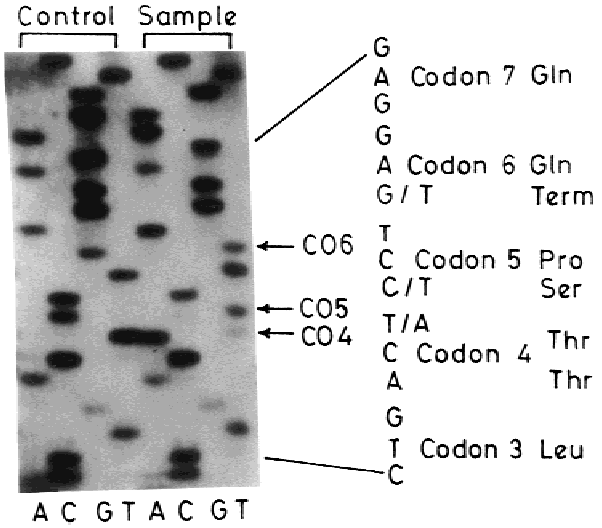

Fig. 1. Pedigree showing the Hematological investigations of the proband and his parents.

Hb	10.1	10.0
MCV	62.0	57.0
OF	60.5	27.5
HbA_2	5.9	6.7
HbF	0.8	1.4
β-globin Mutation	IVS I-5 G→C/N	Co 4 T→A Co 5 C→T Co 6 G→T

N

Fig. 2. Direct sequencing of amplified DNA products from the mother.

DNA sample was subjected to DNA sequencing to delineate the mutation and offer genetic counselling on the couple's demand.

Direct sequencing of amplified DNA products from the mother, using biotin-labelled primer, revealed three point mutations on the sense strand at codons 4–6. The mutation of T→A at codon 4 did not change the amino acid, while mutations C→T at codon 5 and G→T at codon 6 led to a change of amino acid from proline to serine and glutamine to termination codon, respectively. These are new mutations which have not been described earlier in the β-globin gene of any other ethnic group. One base substitution at the 84th codon (ACC→AAC) resulted in an amino-acid change (Thr→Asn), and the other in a two-base substitution at the 85th codon (TTT→AAT) resulting in an amino-acid change (Phe→Asn) located on the same chromosome, was reported by Kim et al. [8]. Hb Poissy [9] is another β-globin chain variant with double substitution (codon 56 Gly→Arg and codon 86 Ala→Pro), in which two mutations are rare and 90 bases apart within the second exon of the β-gene.

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